Archives of Oral Biology 60 (2015) 1488–1496

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/aob

Protective efficacy of intravenous transplantation of adipose-derived stem cells for the prevention of radiation-induced salivary gland damage

Zhijin Li[a,1], Yan Wang[a,1], Hongyan Xing[b], Zhifa Wang[a], Hanqing Hu[a], Ran An[c], Haiyan Xu[c], Yanpu Liu[a,**], Bin Liu[c,*]

[a] State Key Laboratory of Military Stomatology, Department of Oral and Maxillofacial Surgery, School of Stomatology, Fourth Military Medical University, Xi'an 710032, PR China
[b] Department of Stomatology, Xinzhou 2nd People's Hospital, 120 Yong Xing Nan Road, Xinzhou, Shanxi Province 034100, PR China
[c] State Key Laboratory of Military Stomatology, Laboratory Animal Center, School of Stomatology, Fourth Military Medical University, Xi'an 710032, PR China

ARTICLE INFO

Article history:
Received 24 December 2014
Received in revised form 25 June 2015
Accepted 25 July 2015

Keywords:
Adipose-derived stem cells
Cell therapy
Radiation injuries
Salivary gland

ABSTRACT

Objective: High-dose radiation therapy in the head and neck area can lead to irreversible damage to salivary glands (SGs) with consequent xerostomia. Adipose-derived stem cells (ADSCs) have been shown to repair or rescue damaged SGs. Thus, we investigated the protective efficacy of ADSCs in the prevention of SG damage induced by high dose radiation.
Methods: Third-passage ADSCs (1×10^6) were transplanted by intravenous infusion into the tail-vein of 8-week-old C57BL/6 mice, immediately after local irritation at a dose of 18 Gy. The process was repeated twice a week during a period of six consecutive weeks. Eight weeks after radiation, functional evaluations were conducted by measuring salivary flow rate (SFR). Histological, immunohistochemical and transmission electron microscopic (TEM) examinations were performed to analyze microstructural and ultrastructural changes, microvessel density, amylase production, apoptosis, and proliferation activity.
Results: Intravenously administrated ADSCs could home to irradiated SGs within 24 h after infusion, significantly increasing SG weights, improving SFR, and preserving the microscopic morphologies of SGs eight weeks post-radiation. More functional acini, higher amylase production levels, and higher microvessel densities were observed in ADSC-treated SGs than in irradiated SGs. Additionally, enhanced cell proliferation activity and reduced radiation-induced SG apoptosis was observed in the ADSC-treated group when compared with the irradiated group.
Conclusion: Systemic administration of ADSCs immediately after radiation at a dose of 18 Gy can protect both the morphology and function of SGs eight weeks after radiation in mice, and can be used as a protective measure for the prevention of SG damage induced by high-dose radiation.
© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Radiotherapy is commonly used in the comprehensive treatment for most head and neck cancers. However, it can directly or indirectly result in irreversible damage of the salivary glands (SGs) and consequently decrease saliva production. This may lead to a broad range of symptoms and medical problems such as xerostomia, dysfunction of speech, chewing and swallowing, severe dental caries, dysgeusia and mucositis (Bhide, Miah, Harrington, Newbold, & Nutting, 2009; Vissink et al., 2010). These life-long problems severely reduce the post-treatment quality of life and increase the distress level of a large number of patients receiving radiation in the head and neck region.

* Corresponding author at: State Key Laboratory of Military Stomatology, Laboratory Animal Center, School of Stomatology, Fourth Military Medical University, 145 West Changle Road, Xi'an 710 032, P.R. China. Fax: +86 29 84776175.
** Corresponding author at: State Key Laboratory of Military Stomatology, Department of Oral and Maxillofacial Surgery, School of Stomatology, Fourth Military Medical University, 145 West Changle Road, Xi'an 710032, P.R. China. Fax: +86 29 83224470.
E-mail addresses: liuyanpu@fmmu.edu.cn (Y. Liu), kqyljd_liu@126.com, kqyljd_liu@yeah.net (B. Liu).
[1] These authors contributed equally to this work and should be considered as co-first authors.

http://dx.doi.org/10.1016/j.archoralbio.2015.07.016
0003-9969/© 2015 Elsevier Ltd. All rights reserved.

Most studies describe a threshold dose of about 26–39 Gy in order to achieve a significant reduction in salivary secretion in human adults (Ortholan et al., 2009; Roesink et al., 2001). The predictability of radiation-induced SG damage indicates the need for more active measures during and/or immediately after the earlier phase post-radiation, rather than treating hypofunction until irreversible damage has occurred. Current strategies preventing or minimizing radiation-induced SG damage include the use of protective agents, such as amfostin or tempol, surgical relocation of the submandibular gland, and minimization of the radiation dose delivered to non-target normal SGs (Bhide et al., 2009; Vissink et al., 2010). Nevertheless, a satisfactory preventive therapy has not been devised to protect the SGs from radiation-induced damage.

In recent years, some researchers have investigated cell therapy utilizing different stem cell sources to achieve this goal (Kojima et al., 2011; Lim, Ra et al., 2013; Lim, Yi et al., 2013; Lin et al., 2011; Lombaert et al., 2008; Sumita et al., 2011; Tran et al., 2013; Yamamura et al., 2013). In the past decade, adipose-derived stem cells (ADSCs) have been confirmed to be a readily available, multipotent, and have the capability to regenerate into different tissue lineages, such as bone, cartilage, and SGs (Gir, Oni, Brown, Mojallal, & Rohrich, 2012; Lim, Ra et al., 2013). Only two studies (Kojima et al., 2011; Lim, Ra et al., 2013) have investigated the use of ADSCs for the treatment of radiation-induced SG damage. Kojima et al. (Kojima et al., 2011) found that intraglandular administration of ADSCs 10 weeks after a 5 Gy dose radiation could partly restore SG function in mice. Lim, Ra et al. (2013) explored the effects of systemic infusions of human ADSCs on SG regeneration in mice. Intravenous infusion began within six hours after a 15 Gy dose radiation and was performed weekly for three consecutive weeks. Results from the afore-mentioned study showed that ADSCs could protect SGs from radiation damage in mice. However, whether cell therapy with ADSCs can protect SGs against damage induced by higher dose radiation is unknown.

Our preliminary study (unpublished data) found that 15 Gy decreased 30% of the salivary flow, while about 50–60% of the flow was reduced at a dose of 18 Gy eight weeks after radiation. However, consistent with the results from a study conducted by Sumita et al. (2011), we found that radiation doses higher than 20 Gy were too severe for the health of the mice. Clinically, SG damage induced by radiation usually occurs shortly after radiotherapy. This decreases saliva flow by 50–60% during the first week and finally declines to approximately 20% seven weeks after conventional radiotherapy (Franzen, Funegard, Ericson, & Henriksson, 1992). Furthermore, oral dryness is generally not observed until salivary flow is reduced by at least 50% (Furness, Worthington, Bryan, Birchenough, & McMillan, 2011). Therefore, radiation at a high dose of 18 Gy in mice closely mimics the clinical features of most patients suffering from xerostomia after local radiation, and is an optimal dose to study radiation-induced SG damage in mice.

The aim of this study was to assess the protective efficacy of systemically transplanted ADSCs in protecting SGs against damage induced by high-dose radiation.

2. Materials and methods

2.1. Animals

Eight-week-old female C57BL/6 mice weighing 20–22 g were purchased from the Laboratory Animal Center, at the Fourth Military Medical University in Xi'an, China. The mice were maintained under clean conventional conditions in a temperature-controlled room (24 °C) on a 12-h/12-h light and dark cycle at the animal experimental center. All of the experimental procedures were approved by the Experimental Animal Welfare and Ethics Committee at the Fourth Military Medical University.

2.2. Radiation

The mice were anesthetized with pentobarbital sodium (2%) and aligned in the radiation unit. SG damage was elicited by local radiation with a single-dose exposure of 18 Gy using an electronic linear accelerator (Mevatron MD, Siemens Medical Laboratories Inc., Germany). Non-target areas of the body were protected by a 12-mm-thick lead shield.

2.3. Isolation, culture and characteristics of ADSCs

ADSCs were harvested from the inguinal fat pads of eight-week-old C57BL/6 female mice under general anesthesia. The adipose tissues were washed repeatedly with sterile phosphate-buffered saline (PBS) to remove blood components, and then minced and digested with 0.075% type I collagenase (Sigma–Aldrich, St. Louis, MO, USA) in PBS, under agitation for 60 min at 37 °C. The floating fraction was discarded, and the resultant material was filtered through a 100-μm cell strainer (BD Biosciences, San Jose, CA, USA) and centrifuged at 1500 rpm for 5 min. The cell pellet was re-suspended and then transferred to culture flasks filled with DMEM/F12, 10% fetal bovine serum (FBS, Gibco), 100 units/ml penicillin, and 100 μg/ml streptomycin (Gibco). Non-adherent cells were removed after three days and the remaining adherent cells were considered ADSCs. After attaining 80% confluence, the cells were passaged at a sub-cultivation ratio of 1:2. ADSCs, at passage 3, were used in the experiments. Surface marker expression of ADSCs was assessed by flow cytometry (FACSCalibur, Becton-Dickinson, San Jose, CA). Cells were labeled with monoclonal antibodies against CD29, CD31, CD34, CD44, CD45, and CD90. *In vitro* differentiation into osteogenic, adipogenic, and chondrogenic lineages were evaluated. Briefly, for osteogenic differentiation, ADSCs were cultured in osteogenic media for three weeks, and osteogenesis was assessed by evaluating morphological changes and mineralization using Alizarin red staining. For adipogenic differentiation, ADSCs were cultured in adipogenic media for two weeks, and adipogenesis was assessed by Oil-Red-O staining for the presence of lipid vacuoles, whereas, in the case of chondrogenic differentiation, ADSCs were cultured in chondrogenic media for three weeks, and chondrogenesis was assessed by toluidine blue staining. All staining procedures were performed according to the manufacturer's protocols.

2.4. In vivo *cell homing assays*

In order to determine the homing of intravenously transplanted stem cells to the irradiated SGs, ADSCs were labeled with fluorescent Cell Tracker™ CM-DiI (Invitrogen, Carlsbad, CA, USA). Twelve mice were randomly divided into two different groups (six mice per group) and exposed to: (1) intravenous injections of CM-DiI-labeled ADSCs immediately post-radiation and (2) intravenous injections of CM-DiI-labeled ADSCs without radiation. Third-passage ADSCs were incubated with 2 μg/ml of CM-DiI in DMEM with 10% FBS at 37 °C for 5 min, and then for an additional 15 min at 4 °C. After labeling, ADSCs were washed with PBS and re-suspended in PBS at a concentration of 5×10^6 cells/ml. Then, 200 μl labeled cells were injected intravenously through the tail vein of the mice, and the dose was repeated twice at six hour intervals. Twenty-four hours after the last dose, the mice were sacrificed and the submandibular glands were surgically removed. Tissue samples were immediately embedded in Tissue-Tek optimal cutting temperature (Sakura Finetek), snap-frozen in liquid nitrogen, and stored at −80 °C. Samples were cut into 6-μm

Download English Version:

https://daneshyari.com/en/article/3120780

Download Persian Version:

https://daneshyari.com/article/3120780

Daneshyari.com